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# Glial Cells and Neurotransmission: An Inclusive View of Synaptic Function

Review

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**Glial cells throughout the nervous system are closely associated with synapses. Accompanying these anatomical couplings are intriguing functional interactions, including the capacity of certain glial cells to respond to and modulate neurotransmission. Glial cells can also help establish, maintain, and reconstitute synapses. In this review, we discuss evidence indicating that glial cells make important contributions to synaptic function.**

## Introduction

Glial cells were long believed to be simple support cells for neurons. Given this perception, the revelation that some of these cells engage in dynamic interactions with synapses during neurotransmission—the most quintessential neuronal function—was surprising. Evidence shows that glial cells can (1) respond to neurotransmission, (2) modulate neurotransmission, and (3) instruct the development, maintenance, and recovery of synapses. In fact, many synapses have a glial contribution(s) that modulates information flow between neurons. Based on these criteria, we will discuss how glial cells can affect neurotransmission at synapses; wherever possible, in situ examples will be highlighted. The reader is referred elsewhere for consideration of other neuron-glial interactions, which contribute to glial proliferation, differentiation, myelination, etc. (for reviews, see Barres and Raff, 1999; Fields and Stevens-Graham, 2002).

## Ensheathement of Synapses by Glial Cells

There are a number of common features that characterize the anatomical relationship of glial cells with CNS and PNS synapses, notably the close proximity of glial processes to transmitter release sites (Figure 1). For instance, at vertebrate NMJs, perisynaptic Schwann cells (PSCs) extend processes that run in close opposition to the nerve terminal and encroach upon the synaptic cleft with finger-like extensions near active zones (Figure 1A; for review, see Salpeter, 1987). In fact, it is exceedingly rare for nerve terminals not to be closely associated with PSC processes.

In keeping with the heterogeneity of CNS synapses, the extent of their synapse-glial contact is not uniform. For instance, hippocampal astrocytes differentially contact synapses (Figures 1B and 1C), and most (about 80%) of stratum radiatum large perforated synapses are contacted by astrocytic processes, but only about half of smaller macular synapses are associated with processes (Ventura and Harris, 1999). In contrast, Bergmann glia processes in the cerebellum ensheathe most parallel and climbing fiber synapses (Grosche et al., 1999, 2002; Spacek, 1985), with branches off the main glial process, termed microdomains, encapsulating groups of many synapses (Figure 1D; Grosche et al., 1999, 2002). Retinal Müller cells (Newman and Zahs, 1998), hypothalamic astrocytes (Oliet et al., 2001), hippocampal oligodendrocyte precursor cells (Bergles et al., 2000), and other glial cells are also closely associated with synapses.

The variable physiology of different synapses likely helps establish—and is influenced by—features of glial-synapse contact. In fact, the close proximity of certain glial cells to synapses facilitates their capacity to respond to neurotransmission.

## Glial Cell Activation by Neurotransmission

Glial cells that contact synapses are typically sensitive to synaptic activity. The fundamental importance of this is implied by its evolutionally conservation, with both invertebrate (for example, see Britz et al., 2002) and vertebrate glial cells responding to synaptic activity. Since glial cells are not electrically excitable in the fashion of neurons, information is encoded differently. One important glial cell response to neurotransmission is increased cytosolic $Ca^{2+}$ concentration. As will be discussed in a subsequent section, this is believed to be crucial for some forms of glial-induced modulation of synaptic activity.

PSCs at the frog NMJ respond to high-frequency synaptic activity with increased $Ca^{2+}$ in situ (Figure 2A; Jahromi et al., 1992; Reist and Smith, 1992; for review, see Auld and Robitaille, 2003). This is dependent on neurotransmitter release, and PSCs respond to ACh, ATP, and substance P with increased $Ca^{2+}$ from an intracellular pool(s). Mouse PSCs also respond to synaptic activity with increased $Ca^{2+}$ (Rochon et al., 2001), indicating that this is an evolutionarily conserved characteristic.

Compared to PSCs, many CNS glial cells display similar responses to synaptic activity. Pioneering work showed that glial cells in culture respond to neurotransmitters, and this has been largely confirmed in more intact in situ slice preparations (for reviews, see Araque et al., 2001; Carmignoto, 2000; Verkhratsky et al., 1998). In hippocampal astrocytes in situ, glutamate applications induce $Ca^{2+}$ responses in processes and somata by metabotropic and ionotropic receptors (Pasti et al., 1997; Porter and McCarthy, 1995; Shelton and McCarthy, 1999). Also, $Ca^{2+}$ is increased in hippocampal astrocytes by synaptic activity in acute or cultured slice preparations, which also involves glutamate (Dani et al., 1992; Pasti et al., 1997; Porter and McCarthy, 1996). The

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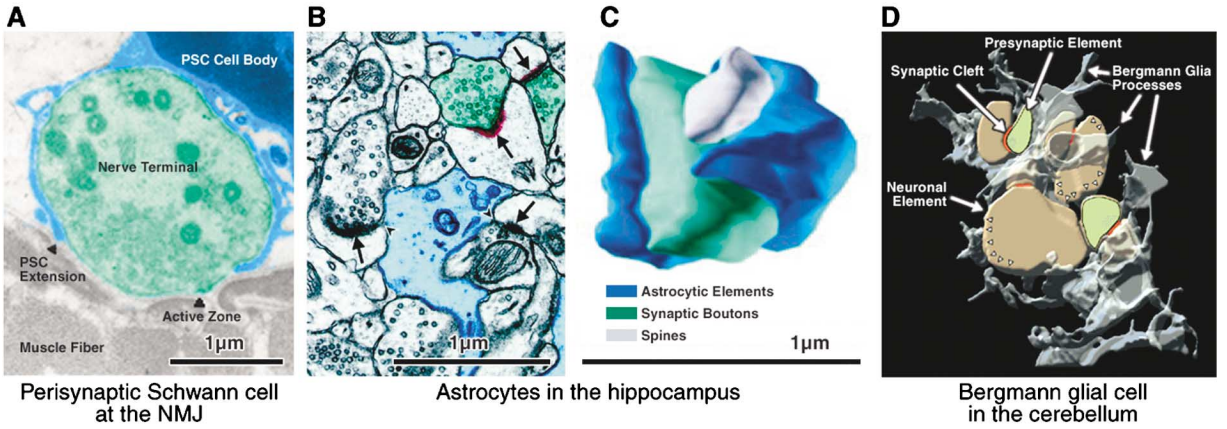


Figure 1. Glial Ensheathement of PNS and CNS Synapses

(A) Finger-like extensions of a PSC at a frog NMJ encroach upon the synaptic cleft. Modified from Jahromi et al. (1992), copyright and reprinted with permission from Elsevier.

(B and C) Micrograph and reconstruction of synapses (arrows) in the stratum radiatum of the CA1 area of the hippocampus: astrocytic processes (arrowheads) are evident adjacent to pre- and postsynaptic elements at certain synapses. Modified from Ventura and Harris (1999), copyright by the Society for Neuroscience.

(D) Reconstruction of a group of neighboring cerebellar synapses with surrounding Bergmann glial cell (blue-green). Small arrowheads point to neuronal surfaces not covered by glial sheaths from the labeled cell. Modified from Grosche et al. (2002), copyright and reprinted by permission of Wiley-Liss Inc., subsidiary of John Wiley & Sons, Inc.

astrocyte $Ca^{2+}$ response is characterized by repetitive elevations, and the frequency of these relatively slow (versus presynaptic firing) oscillations rises with the frequency of synaptic activity (Pasti et al., 1997). Recently, the astrocyte $Ca^{2+}$ oscillation response has been shown to contribute to arteriole dilation, suggesting its importance for coupling neuronal activity to blood flow (Zonta et al., 2003).

Hippocampal astrocytes also respond to adenosine, ATP, GABA, histamine, norepinephrine, and acetylcholine (for review, see Verkhratsky et al., 1998). This wide array of neurotransmitters is consistent with the neurochemical heterogeneity of hippocampal innervation; the possibility that astrocytes could contribute to the neuromodulatory action of these inputs is an exciting prospect. In keeping with this, inhibition of astrocyte glutamate uptake has been shown to be involved in adenosine-induced potentiation of synaptic efficacy in hippocampal cultures (Nishizaki et al., 2002).

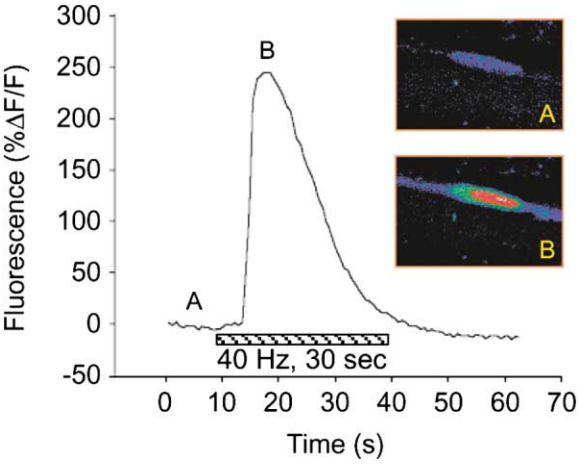


Figure 2. A PSC at the Frog NMJ Responds to Synaptic Activity with Increases in $Ca^{2+}$

Data from the authors' laboratory.

Bergmann glia of the cerebellum also respond to neurotransmitters with cytosolic $Ca^{2+}$ increases (for review, see Verkhratsky et al., 1998). Glutamate acts on Bergmann glia through AMPA receptors that have high levels of $Ca^{2+}$-permeable GluR1 and GluR4 subunits (Burnashev et al., 1992; Muller et al., 1992), but nonionotropic receptors linked to intracellular $Ca^{2+}$ stores may also be involved (Kirischuk et al., 1999). Parallel fiber stimulation increases $Ca^{2+}$ in Bergmann glia microdomains (Grosche et al., 1999), and this has been shown to depend on nitric oxide (Matyash et al., 2001).

Nevertheless, glutamate signaling by AMPA receptors likely plays an important role in the maintenance of Purkinje cell spine synapse ensheathement by Bergmann glia. Eliminating the $Ca^{2+}$ permeability of these receptors results in process retraction, leaving synapses naked (Figure 3; Iino et al., 2001). This is accompanied by delayed removal of synaptic glutamate (Iino et al., 2001). Thus, rather than engaging in static encapsulation, Bergmann glia appear to require constant input from their associated synapses to maintain the relationship. In this fashion, active synapses likely help instruct their own glial ensheathement, which subsequently influences their function by changes in glutamate uptake (Iino et al., 2001). In fact, glutamate uptake is a major mechanism by which many CNS glial cells influence synaptic performance.

## Modulation of Neurotransmission by Glial Glutamate Uptake

Glial ensheathement is important for isolating synapses from one another. Glial processes (notably hippocampal astrocytes and Bergmann glia) have high concentrations

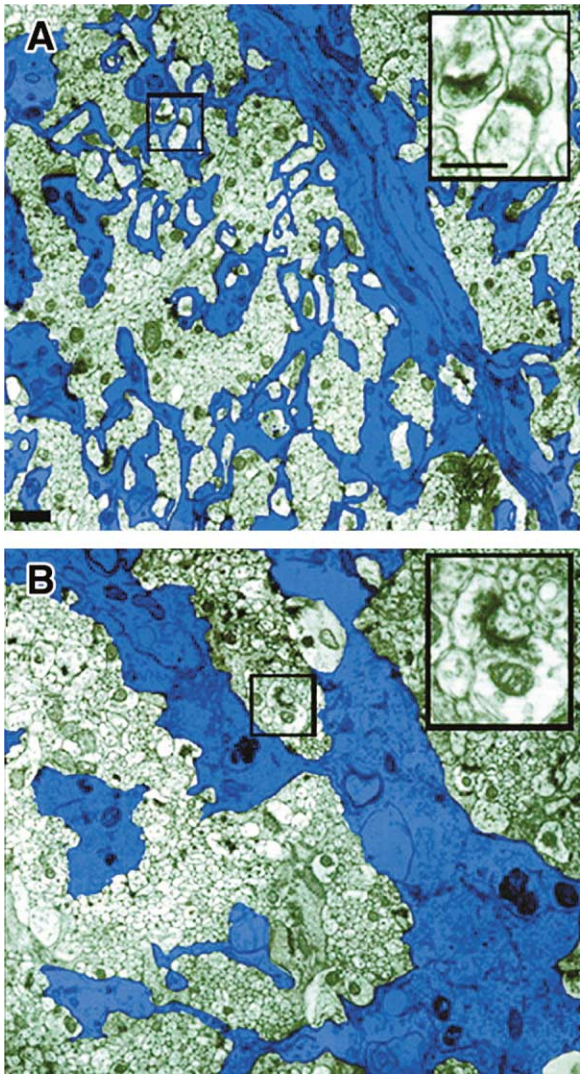


Figure 3. Glutamatergic Signaling Helps Maintain Glial Ensheathment of Cerebellar Synapses

Disruption of AMPA receptor signaling in Bergmann glia (blue) by adenoviral delivery of $Ca^{2+}$-impermeable GluR2 subunits is accompanied by loss of synapse encapsulation: $Ca^{2+}$-permeable (A) and -impermeable (B) AMPA receptors. Scale bars represent 1 μm in (A) and (B) and 0.5 μm in the inserts. Reprinted with permission from Iino et al. (2001), copyright American Association for the Advancement of Science.

of GLAST and GLT-1 glutamate transporters, with uptake and/or binding of glutamate by these transporters being critical for limiting the action of glutamate at many synapse (Bergles and Jahr, 1997; Chaudhry et al., 1995; Clark and Barbour, 1997; Lehre and Danbolt, 1998; Rothstein et al., 1996; Rusakov and Kullmann, 1998; for review, see Danbolt, 2001). Indeed, recordings of transport currents from hippocampal astrocytes and pyramidal neurons show that astrocytes are principally responsible for glutamate clearance at certain hippocampal synapses (Bergles and Jahr, 1998). Bergmann glia also respond to stimulation of the granule cell layer and parallel fibers with glutamate uptake (Bergles et al., 1997; Clark and Barbour, 1997), and GLAST is concentrated proximal to Purkinje cell synapses (Chaudhry et al., 1995). The probable importance of this for establishing characteristics of synaptic transmission has been demonstrated by kinetic modeling (Rusakov and Kullmann, 1998), as well as by observations showing that glutamate transporters influence synaptic efficacy (Tong and Jahr, 1994) and glutamate spillover affects neurotransmission at neighboring synapses (Mitchell and Silver, 2000). Together, these studies imply that uptake and/or binding of glutamate by these glial transporters helps to determine the flow of information within both hippocampal and cerebellar circuitries. This is likely also the case in the retina (Lehre et al., 1997) and hypothalamus (Oliet et al., 2001).

Interestingly, a physiologically occurring process in the hypothalamic supraoptic nucleus depends on synapse contact and glutamate uptake by astrocytes (Oliet et al., 2001). During lactation, astrocytic processes withdraw from synapses and this is associated with increases in glutamate concentration and/or clearance time at these synapses. This is hypothesized to enhance inhibitory feedback through presynaptic metabotropic glutamate receptors, subsequently decreasing the probability of release (Oliet et al., 2001). Thus, changes in systemic physiology (lactation) are associated with changes in the proximity of glial processes to synapses, which in turn have functional consequences for synaptic efficacy.

In light of the association between changes in synapse contact by glia and changes in synaptic function (Iino et al., 2001; Oliet et al., 2001), the disparity of synapse coverage by glial cells within and between CNS regions is intriguing (Spacek, 1985; Ventura and Harris, 1999). It is possible that synapses that are differentially associated with glial processes may also behave differently by virtue of that association. If glial coverage is capable of remodeling, this could be important for changing the character of glial neuromodulation and could contribute to certain forms of synaptic plasticity. In this regard, it may be telling that induction of LTP (Wenzel et al., 1991) and exposure to a complex environment (Jones and Greenough, 1996) enhance contacts between astrocyte processes and synaptic elements. Indeed, physiological or pathological factors that influence glial synapse coverage, glutamate uptake, and/or changes in secreted neuromodulators (see below) are likely to influence synapse behavior and information processing within many neuronal circuits.

New evidence links glial glutamate uptake with a classically envisioned glial function, namely the provision of metabolites to neurons (Voutsinos-Porche et al., 2003). It is hypothesized that $[Na^+]_i$ increases, which accompany glutamate uptake, enhance astrocyte glucose utilization and lactate secretion, the latter of which can be used by neurons as an energy substrate. Thus, glutamate uptake by glial cells can serve as a precise trigger for supplying energy to synapses when and where it is most needed. This complements the importance of astrocyte $Ca^{2+}$ oscillations for increasing blood flow (Zonta et al., 2003), implying that different glial responses to neurotransmission coordinate the local supply of energy and blood flow in an activity-dependent fashion.

Another contribution of glial cells to glutamate neurotransmission is precursor supply. Following uptake by

astrocytes, glutamate is converted to glutamine and subsequently returned to nerve terminals for processing back into glutamate (for review, see Hertz et al., 1999). In keeping with its significance for synaptic function, pharmacological disruption of this pathway alters glutamatergic neurotransmission (Bacci et al., 2002). Thus, through glutamate uptake and glutamine shunt, certain glial cells have a dual influence on neurotransmission. However, glial cells are increasingly being recognized as modulating synaptic activity through other means as well.

### Glia Modulation of Neurotransmission and Neuronal Activity by Secreted Molecules

In this section, we present evidence showing that certain glial cells can influence neuronal excitability and neurotransmission by secreted neuromodulators. In many cases, this appears to be dependent on glial $Ca^{2+}$ increases, showing that this well-documented response to neurotransmission is critical for neuromodulatory feedback. These studies bolster the concept of the tripartite synapse, which posits that certain glial cells serve as integral modulatory elements at synapses (Araque et al., 1999).

Pioneering work by Haydon and colleagues, using hippocampal neuron-glial cocultures, has shown that activation of astrocytes with bradykinin subsequently increases $Ca^{2+}$ in neighboring neurons in an NMDA-dependent fashion, implying that activated astrocytes release glutamate (Parpura et al., 1994). Subsequent work has shown that astrocyte $Ca^{2+}$ increases in response to stimulation (e.g., mechanical) have three major effects on neighboring neurons: (1) induction of a slow postsynaptic NMDA-dependent inward current; (2) an increase in TTX insensitive spontaneous neurotransmitter release from excitatory and inhibitory neurons; and (3) a decrease in action potential-evoked excitatory and inhibitory postsynaptic currents, likely involving activation of presynaptic metabotropic glutamate receptors (Araque et al., 1998a, 1998b). In these studies, the use of $Ca^{2+}$ chelators and $Ca^{2+}$ photolysis has shown that $Ca^{2+}$ elevation in astrocytes is necessary and sufficient for neuromodulation. Vesicular proteins are present in astrocytes and are believed to be important for glutamate release (Araque et al., 2000). Together, these experiments show that astrocytes can acutely modulate neuronal activity and synaptic transmission by activating various neuronal glutamate receptor subtypes in a fashion that is likely to involve regulated secretion of glutamate or an agonist.

Complementary to glutamate receptor activation following astrocyte stimulation, cultured hippocampal astrocytes can secrete the novel neuromodulator D-serine. In keeping with the well-characterized sensitivity of astrocytes to glutamate, secretion of D-serine can be induced by exposure to this neurotransmitter, likely through kainate receptor activation (Schell et al., 1995). Interestingly, D-serine is a potent agonist for the glycine binding site of NMDA receptors (Mothet et al., 2000), raising the possibility that some of the effects ascribed to glial glutamate release could also involve glial D-serine release (see below). Moreover, it was shown that serine racemase, the D-serine synthesis enzyme, is selective for glial cells (Wolosker et al., 1999). Consistent with a possible role in vivo, D-serine is enriched in astrocyte processes and colocalized near NR2A/B subunits (for review, see Snyder and Kim, 2000) at known sites of astrocyte neuromodulation.

Glial modulation of neuronal activity and neurotransmission is not a feature that is only observed in culture conditions. Indeed, astrocyte activation is associated with neuromodulation in neuronal circuits in situ. In hippocampal slices, astrocyte $Ca^{2+}$ elevations elicited by repetitive neuronal activity or exposure to metabotropic glutamate receptor antagonists are followed by delayed $Ca^{2+}$ elevations in neighboring neurons, which are glutamate receptor dependent and tetanus toxin resistant (Pasti et al., 1997). Interestingly, evidence suggests that prostaglandins (notably $PGE_2$) stimulate astrocyte glutamate release in a $Ca^{2+}$-dependent fashion. Moreover, the release of glutamate by astrocytes following their activation via AMPA/kainate and metabotropic receptors is prevented by prostaglandin synthesis inhibitors (Bezzi et al., 1998). Interestingly, the prostaglandin/astrocytic glutamate release pathway participates in chemokine-induced neurotoxicity (Bezzi et al., 2001). These data suggest that neuron-glial interactions could contribute to pathological conditions in the nervous system.

As is the case for $Ca^{2+}$ responses and glutamate uptake, Bergmann glia resemble hippocampal astrocytes in their capacity to modulate neighboring neurons through glutamatergic receptor activation. Direct depolarization of Bergmann glial cells in situ results in long-lasting reduction of the frequency of miniature postsynaptic currents recorded in Purkinje neurons (Brockhaus and Deitmer, 2002). This is associated with activation of ionotropic non-NMDA receptors, consistent with the involvement of glutamate as a glial-derived neuromodulator in situ. Moreover, as with hippocampal astrocytes (see above) and Müller glia (see below), D-serine is present in Bergmann glia and may be used as a neuromodulator (for review, see Snyder and Kim, 2000).

In addition to the hippocampus and the cerebellar cortex, regulated glial secretion of neuroactive glutamate also appears to occur in the retina. In situ, mechanical stimulation of glial cells induces $Ca^{2+}$ waves that propagate through retinal glial cells (Newman and Zahs, 1998; for discussions of glial $Ca^{2+}$ waves, see Araque et al., 2001; Carmignoto, 2000; Verkhratsky et al., 1998). When these waves pass retinal ganglion neurons, they cause changes in light-evoked spike frequency (usually reductions, but also increases) (Figure 4). The extent of this modulation is proportional to the glial $Ca^{2+}$ elevation, and treatments that reduce $Ca^{2+}$ also reduce the effect on spike frequency. The reduction of spike frequency probably involves activation of inhibitory interneurons following glutamate release from glial cells in a $Ca^{2+}$-dependent fashion. This is supported by the observations that antagonism of AMPA and NMDA receptors reduced the inhibition of spike activity. In addition to glutamatergic mechanisms, ATP release by activated glial cells also contributes to the inhibition of neuron spiking, with this purinergic modulation likely originating selectively from Müller cells and involving adenosine produced by ATP degradation (Newman, 2003). In summary, neuron activity induced by the natural stimulus

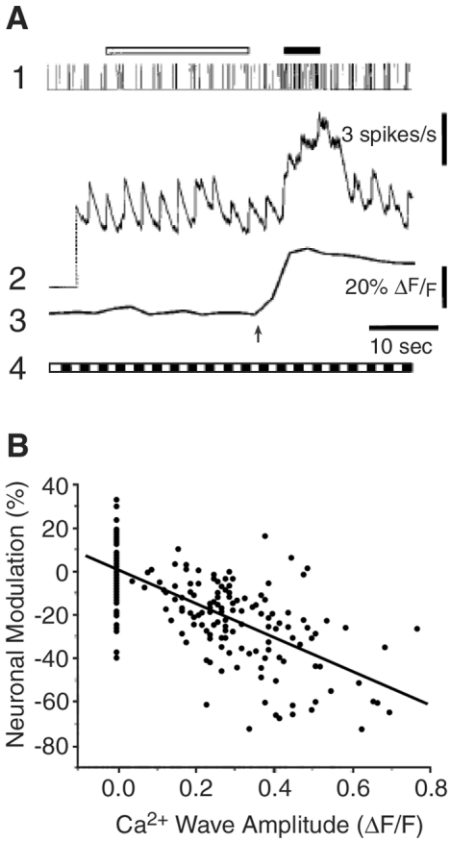


Figure 4. Modulation of Neuronal Activity in the Retina

(A) Changes in neuronal firing of an ON cell (lane 1, action potentials; lane 2, frequency plot) induced by alternating on and off light stimulus (lane 4) before and during a glial $Ca^{2+}$ wave (lane 3) mechanically induced at the arrow.

(B) Reverse relationship between modulation of neuronal activity (spike frequency) and amplitude of $Ca^{2+}$ waves. Modified from Newman and Zahs (1998), copyright by the Society for Neuroscience.

(light) can be reduced by $Ca^{2+}$-dependent activation of adjacent glial cells in a fashion that is related to the release of neuroactive substances by these cells.

Interestingly, D-serine and its synthesis enzyme have recently been identified in retinal astrocytes (Stevens et al., 2003). Treatment with D-serine enhances light-evoked NMDA currents from ganglion cells. Moreover, disruption of endogenous D-serine reduces tonic- and light-evoked NMDA currents, suggesting that this glial-derived neuromodulator is normally involved in regulating ganglion cell excitability (Stevens et al., 2003).

The growing body of evidence showing that glial-derived D-serine can modulate NMDA currents implies that glutamate released by glial cells may not be solely responsible for effects on NMDA currents at the various synapses where this pattern has been identified (e.g., hippocampus, cerebellum, retina). In fact, it is possible that glial cells modulate NMDA currents by two complementary molecules, namely glutamate and D-serine. It will be interesting to determine the relative importance of these mechanisms, which may differ according to tissue type and developmental stage.

Most of the studies discussed thus far have used direct stimulation of glial cells to evoke neuromodulatory responses. Another important question is whether synaptic activity can activate glial cells to influence the neuronal elements they surround.

Experiments by Nedergaard and colleagues in hippocampal slices have provided evidence in favor of this (Figure 5; Kang et al., 1998). Repetitive firing of interneurons reduces the probability of synapse failure, with this being dependent on $GABA_B$ receptors. Interestingly, chelation of astrocyte $Ca^{2+}$ abolishes this effect, and activation of glial cells (indicated by increased $Ca^{2+}$) by interneuron firing is prevented by $GABA_B$ antagonists. These data suggest that astrocyte activation by interneurons potentiates inhibitory synaptic activity.

Further evidence for glial modulation of synaptic activity has come from the NMJ. Injection of GTPγS into PSCs to activate G proteins (mimicking activation of G protein-coupled receptors) reduced neurotransmitter release evoked by low-frequency motor neuron stimulation (Figures 6A and 6B; Robitaille, 1998). Under these conditions of low-frequency activity, neurotransmission is not altered by GDPβS inhibition of G proteins, suggesting that PSCs do not modulate neurotransmission tonically. However, synaptic depression associated with high-frequency nerve stimulation is reduced by blockade of PSC G protein signaling (Figure 6C). This suggests that, following activation by high levels of synaptic activity at this synapse, perisynaptic glial cells can contribute to the resultant depression of neurotransmitter release.

PSC $Ca^{2+}$ has been evaluated under similar conditions to determine whether this well-characterized PSC response to synaptic activity contributes to feedback neuromodulation. Surprisingly, in contrast to GDPβS, chelation of $Ca^{2+}$ with BAPTA injection into PSCs enhances depression accompanying high-frequency stimulation. These data suggest that PSCs potentiate neurotransmitter release in a $Ca^{2+}$-dependent fashion (Figures 6D and 6E; Castonguay and Robitaille, 2001). Consistent with this, direct injection of $IP_3$ into PSCs and thapsigargin exposure, which elevate $Ca^{2+}$, also increase neurotransmitter release (Figure 6D).

The apparent contradiction between the capacity of PSCs to coincidently enhance and diminish neurotransmission in an activity-dependent fashion is in keeping with short-term plasticity at this synapse, which is characterized by an overlap of potentiating and depressing processes (discussed in relation to PSCs by Auld and Robitaille, 2003). Since PSCs are not physically coupled to other synaptic elements, their capacity to modulate neurotransmission is presumably related to secretion of as-of-yet unidentified neuromodulators. In summary, these studies favor the existence of a synapse-glial-synapse regulatory loop, indicating that activation of perisynaptic glial cells by synaptic activity can be accompanied by feedback modulation of synaptic efficacy and short-term plasticity.

Cultures of mollusc neurons and glial cells offer evidence for another synapse-glial-synapse regulatory loop involving a novel mechanism. At these synapses, the presence of glial cells specifically inhibits cholinergic transmission (Smit et al., 2001). In response to neuronal activation by nicotinic receptors, glial cells release a soluble ACh binding protein with homology to nicotinic

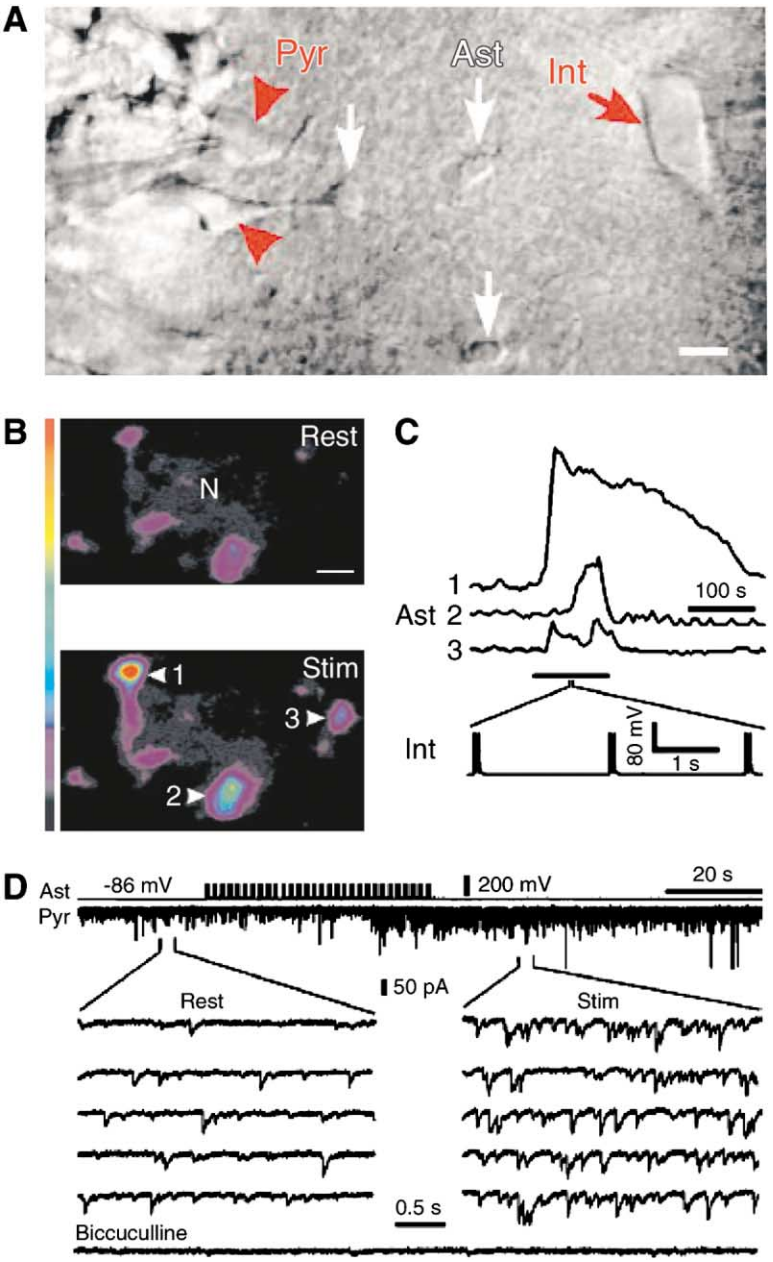


Figure 5. Astrocytes Modulate Inhibitory Synapses in the Hippocampus

(A) Pyramidal neurons (Pyr), interneurons (Int), and astrocytes (Ast) visualized with differential interference contrast.

(B and C) $Ca^{2+}$ responses in astrocytes elicited by activation of an interneuron.

(D) Increase in inhibitory miniature postsynaptic currents following astrocyte stimulation. Modified from Kang et al. (1998) and reproduced with permission and copyright (1998) of Nature Publishing Group.

receptors that buffers ACh, resulting in activity-dependent negative feedback on synaptic efficacy. It will be interesting to determine whether the regulated secretion of comparable buffering proteins could influence neurotransmission at other synapses.

Other evidence suggests that glial cells may contribute to more complex forms of plasticity, such as LTP, learning, and memory (Gerlai et al., 1995; Nishiyama et al., 2002). Moreover, given the importance of AMPA receptors in LTP induction, it is interesting that astrocyte-derived tumor necrosis factor $\alpha$ (TNF$\alpha$) rapidly increases surface expression of neuronal AMPA receptors, resulting in increased synaptic strength (Beattie et al., 2002). In light of the proximity of astrocyte processes to hippocampal synapses, the authors indicate that this implies a mechanism by which glial cells could modulate LTP and LTD. Indeed, rapid and/or prolonged changes in TNF$\alpha$ secretion by astrocytes could result in alterations of synapse behavior. Moreover, considering the key role for NMDA receptors in LTP, it will be interesting to determine if glial cells could influence this process by D-serine secretion.

Complementary to potential glial contributions to neuronal plasticity, glial cells themselves exhibit plasticity in response to repeated stimulation. In fact, changes in astrocyte $Ca^{2+}$ oscillations are associated with repetitive exposure to glutamate (Pasti et al., 1995). Thus, astrocytes exhibit a form of cellular memory that could have significance for the regulation of neuronal properties (Carmignoto, 2000).

Although the studies discussed thus far support the hypothesis that glial modulation of neurotransmission

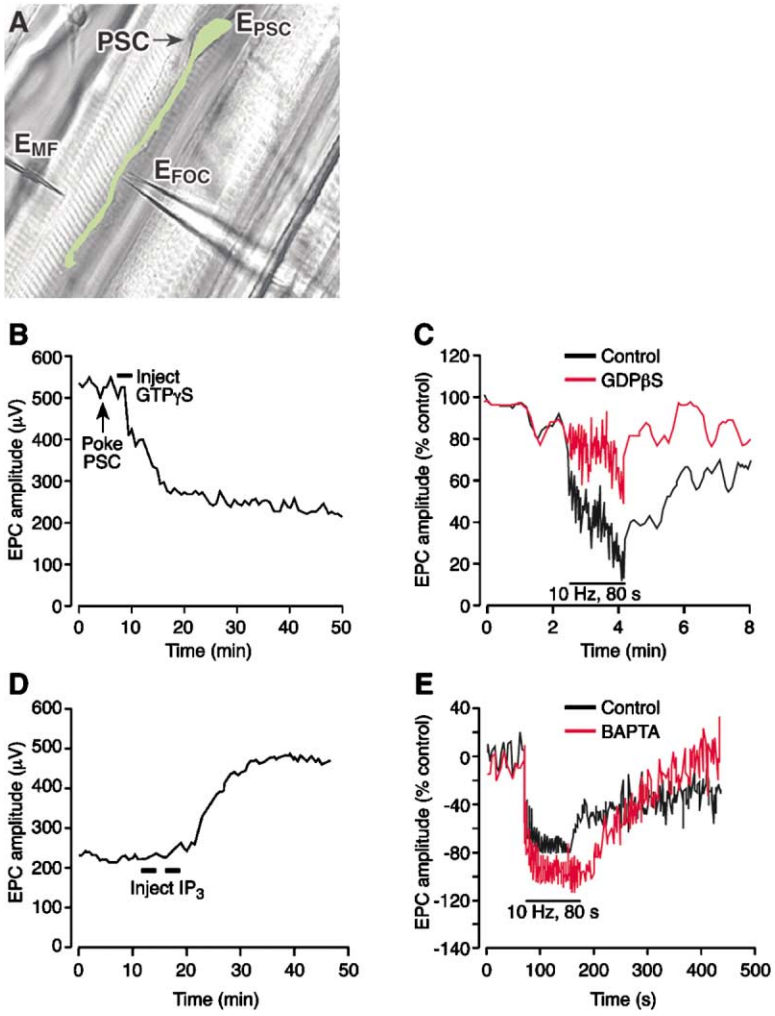


Figure 6. PSCs Modulate Synaptic Plasticity at the Amphibian NMJ

(A) Transmitted light image of an NMJ with three recording electrodes. Superimposed is a fluorescent image of a PSC injected with a $Ca^{2+}$ indicator.

(B) Injection of GTPγS in a PSC reduces transmitter release.

(C) Blockade of PSC G proteins with GDPβS reduces synaptic depression.

(D) Injection of $IP_3$ in a PSC increases transmitter release.

(E) Chelation of PSC intracellular $Ca^{2+}$ increases synaptic depression.

(A)–(C) were modified from Robitaille (1998), (copyright and reprinted with permission from Elsevier), and (D) and (E) are from Castonguay and Robitaille (2001) (copyright by the Society for Neuroscience).

is typically a reciprocal response to synaptic activity, other studies show that neuronal activity can be modulated by glial cells in the absence of obvious neuronal priming. In fact, astrocyte $Ca^{2+}$ oscillations have been observed independently of neuron activation in situ (Aguado et al., 2002; Nett et al., 2002; Parri et al., 2001). In light of this, it is hypothesized that glial cells may act as pacemakers modulating neuronal activity (Nett et al., 2002; Parri et al., 2001). Moreover, activity in astroglial and neuronal networks is correlated (Aguado et al., 2002). Similar to stimulated glial $Ca^{2+}$ waves in the retina, spontaneous propagation of a $Ca^{2+}$ wave between neighboring astrocytes in thalamic slices is associated with an NMDA-dependent inward current in neurons located along the wave path (Parri et al., 2001). These data suggest that glial cells may shape neuronal activity as a response to signaling within glial communication circuits, in addition to doing so as a response to synaptic activity. The existence of glial communication networks based on intercellular $Ca^{2+}$ waves has been discussed elsewhere (Araque et al., 2001; Carmignoto, 2000; Verkhratsky et al., 1998).

In summary, the evidence presented in this section shows that perisynaptic glial cells in the CNS and PNS, in vertebrates and invertebrates, sculpt synaptic efficacy and synaptic plasticity. The direct functional association between neurons and glial cells implies that neuronal networks continually interact with glial cells and even glial networks. We believe that these communication networks may in fact work cooperatively as a unified "neuro-glial" network. In this model, information traveling through the neuronal network can be influenced by glial cells responding to local activity at synapses (reciprocal modulation) or by glial cells responding to activity in glial networks (extrinsically introduced modulation). In both cases, the synapse serves as a significant portal for neuromodulation. Hence, factors that alter glial secretion of neuromodulators—whether resulting from local changes at the synapse or from responses to changes in possible glial networks—or glial-synapse contact are likely to change the flow of information within neuronal circuits.

In addition to these neuromodulatory effects, other studies have revealed quite unexpected effects of glial cells on synapses that are more chronic in nature.

### Glial Cells Influence the Regeneration and Development of Synaptic Contacts

Exciting observations have shown that without glia, some synaptic communication would be much less effi-

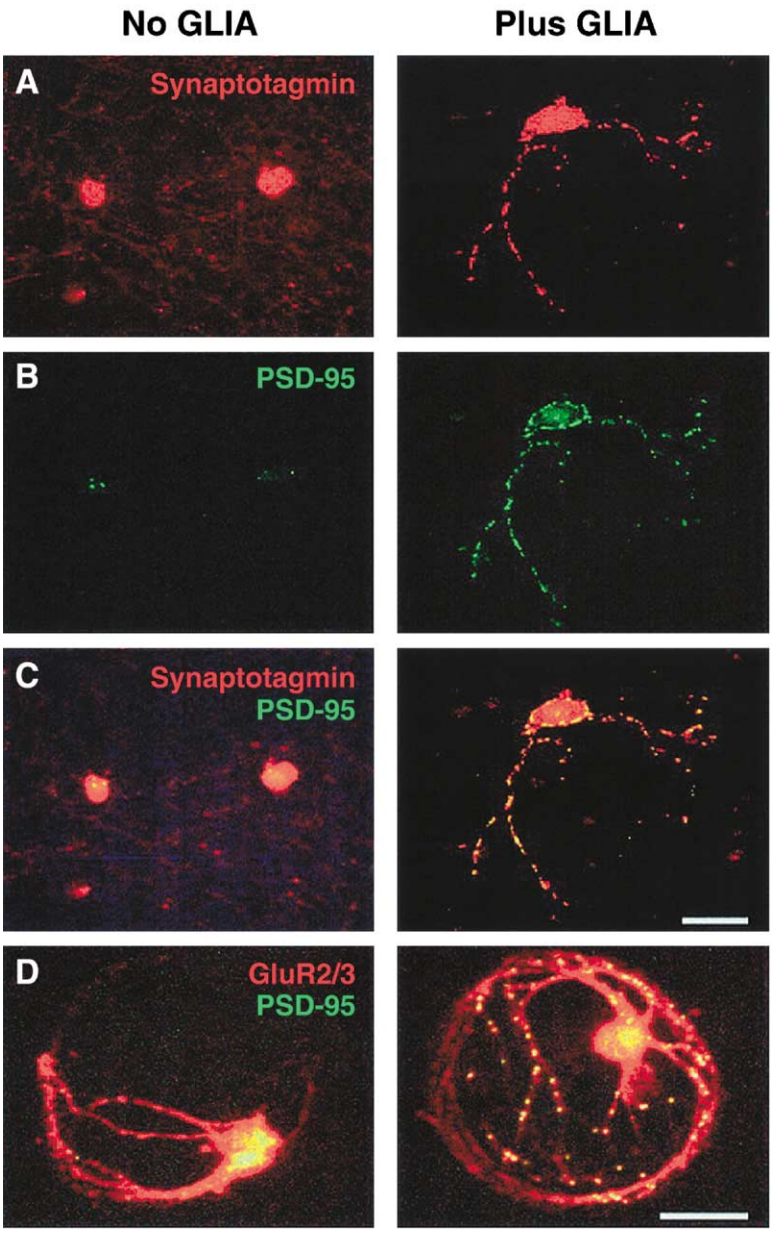


Figure 7. Exposure to Astrocytes Increases Several Synaptic Markers in Retinal Ganglion Cells, as Demonstrated by Immunostaining

Bar represents 50 μm. Reprinted with permission from Ullian et al. (2001), copyright American Association for the Advancement of Science.

cacious. The implications are far reaching, suggesting that glial cells could be vital participants in synaptic development, maintenance, and recovery.

The first indication that neuron-glial relations can be important for re-establishing synapses came from the NMJ (Son and Thompson, 1995a, 1995b). Following muscle denervation, PSCs (also termed terminal Schwann cells) extend process bridges that link endplates and guide regenerating axons to denervated endplates (Koirala et al., 2000; Love and Thompson, 1999; O'Malley et al., 1999; Son and Thompson, 1995a, 1995b). In addition to this clear role in recovery from injury, PSC processes appear to lead innervating neurite sprouts during development as well (Herrera et al., 2000). It is not known if CNS glial cells could similarly guide neurons, but the propensity of astrocytes and oligodendrocytes to inhibit regenerating axons argues against this under many circumstances.

Nevertheless, neurite guidance is not the only fashion by which glial cells can influence synapse development. In fact, retinal ganglion cell synaptic markers are specifically increased by coculture with astrocytes or oligodendrocytes in a fashion that is independent of cell survival and is mimicked by astrocyte-conditioned medium (Figure 7; Mauch et al., 2001; Nagler et al., 2001; Pfrieger and Barres, 1997; Ullian et al., 2001). The effects include enhancement of spontaneous excitatory postsynaptic current (EPSC) frequency, EPSC amplitude, quantal content, and synapse number (Nagler et al., 2001; Pfrieger and Barres, 1997; Ullian et al., 2001). Interestingly, cholesterol/apolipoprotein E complexes appear to contribute to the synapse-promoting effects (Mauch et al., 2001). Since withdrawal of conditioned medium reduces synaptic markers, this indicates that astrocytes are also important for synapse maintenance (Ullian et al., 2001). A role for glial cells in synapse development in vivo is

supported by the observation that synapse formation in the superior colicullus does not occur immediately upon innervation by retinal ganglion cells but is concurrent with the arrival of astrocytes several days later (Ullian et al., 2001). In keeping with the retinal ganglion cell data, recent evidence implies that Schwann cell-derived factors can encourage synaptogenesis in neurotrophin-treated neuron-muscle cell cocultures (Peng et al., 2003), and it is known that PSCs are present at developing NMJs in vivo (Herrera et al., 2000).

Glial cells can alter neurons in another fashion that could have implications for synapse development. In culture, astrocyte contact increases the N-type current in hippocampal neurons during an important period of in vitro synapse formation (Mazzanti and Haydon, 2003). Since this is likely to promote neurotransmitter release, the authors suggest that this could confer an advantage during synapse elimination. In fact, the potential involvement of glial cells in synapse elimination is supported by the observations that factors that influence neurotransmission also modulate synaptic elimination (Katz and Shatz, 1996; Personius and Balice-Gordon, 2002) and that glial cells help determine synaptic efficacy. In addition, the maintenance and promotion of synapses by glia, which is not related to cell survival (Pfrieger and Barres, 1997), is in keeping with the fact that synapse elimination often involves pruning of connections, not death of the parent neuron.

Other tantalizing clues suggest that glial cells help control the extent of innervation at certain synapses. Loss of synapse encapsulation by Bergmann glia is followed by multiple innervation (Iino et al., 2001), implying that they help stabilize successful innervations and/or keep superfluous innervations at bay. Moreover, disruption of GLAST glutamate uptake (a major function of Bergmann glia) is accompanied by an increase in the persistence of multiple innervation in adulthood (Watase et al., 1998). The relevance of these studies is reinforced by the fact that Bergmann glia processes, Purkinje dendrite differentiation, and synapse formation develop concurrently (Yamada et al., 2000). Additional evidence comes from the hypothalamic supraoptic nucleus, where astrocyte processes appear to limit the extent of innervation (for review, see Hatton, 1997).

## Glial Cells, Synapses, and Neuropathology

Based on the numerous interactions between neurons and glial cells at synapses, it seems probable that glial cells may make contributions to some or many of the neurological pathologies that involve altered neurotransmission. In this regard, it is interesting that reduced expression of glutamate transporters results in increased extracellular glutamate concentrations, frequent seizures, and neuronal degeneration (Rothstein et al., 1996; Tanaka et al., 1997). Moreover, glutamate uptake (a major glial function) is sensitive to various insults (e.g., oxidative stress [Volterra et al., 1994]) and has been hypothesized to contribute to several CNS pathologies, including Alzheimer's disease, amyotrophic lateral sclerosis, ischemic insult, and trauma (Danbolt, 2001; Maragakis and Rothstein, 2001; Trotti et al., 1998; Volterra et al., 1994). Based on their contributions to neurotransmission, glial cells could also present novel therapeutic targets. For instance, pharmacological disruption of glutamine synthesis (a major glial function) reduces epileptiform activity, suggesting potentially a novel avenue for epilepsy treatment (Bacci et al., 2002). As discussed in the previous section, glial cells make important contributions to synapse formation, integrity, and maintenance. Assuming that these functions are generally important for synapses beyond the model systems in which they have been identified thus far, it is of great interest to determine whether they are perturbed in—or can be harnessed to ameliorate—neurodegenerative diseases that involve significant disruption of neurotransmission and synapse loss, such as Alzheimer's disease (Auld et al., 2002).

## Concluding Remarks

Glial cells can influence synapses in at least two major fashions: by modulating neurotransmission in the short term and by helping to establish efficacy in the long term. The facts that glial ensheathement of synapses and neurotransmitter responsiveness are plastic suggest that glial influences on neurotransmission may be subject to the kind of dynamic modifications previously believed to apply only to neurons. By virtue of their integration in neuronal circuits, certain glial cells can modify information exchange between neurons, whether prompted by local synaptic activity or signaling in glial communication circuits. The recognition that glial cells could contribute to disruption of neurotransmission in disease states promises novel avenues of insight into their etiology and treatment. In light of the evidence collected by many groups and discussed in this review, we believe that an inclusive view of synaptic function, which considers interactions with glial partners, will furnish a more complete understanding of neurotransmission in relation to physiological and pathological conditions.

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